

Claims

1           1. Substantially pure DNA encoding an *Rps*  
2           polypeptide.

1           2. The DNA of claim 1, wherein said DNA contains  
2           the *RPS2* gene.

1           3. The DNA of claim 1, wherein said DNA is genomic  
2           DNA.

1           4. The DNA of claim 1, wherein said DNA is cDNA.

1           5. The DNA of claim 1, wherein said DNA is of a  
2           plant of the genus *Arabidopsis*.

1           6. Substantially pure DNA having the sequence of  
2           Fig. 2, or degenerate variants thereof, and encoding the  
3           amino acid sequence of open reading frame "a" of Fig. 2.

1           7. Substantially pure DNA having about 50% or  
2           greater sequence identity to the DNA sequence of Fig. 2.

1           8. The DNA of claim 1 or 2, wherein said DNA is  
2           operably linked to regulatory sequences for expression of  
3           said polypeptide; and

4           wherein said regulatory sequences comprise a  
5           promoter.

1           9. The DNA of claim 8, wherein said promoter is a  
2           constitutive promoter.

1           10. The DNA of claim 8, wherein said promoter is  
2 inducible by one or more external agents.

1           11. The DNA of claim 8, wherein said promoter is  
2 cell-type specific.

1           12. A cell which contains the DNA of claim 1.

1           13. The cell of claim 12, said cell being a plant  
2 cell.

1           14. The plant cell of claim 13, said plant cell  
2 being resistant to disease caused by a plant pathogen  
3 carrying an avirulence gene generating a signal recognized  
4 by an Rps polypeptide.

1           15. The plant cell of claim 14, said plant pathogen  
2 carrying an *avrRpt2* gene.

1           16. The plant cell of claim 14, said plant cell  
2 being from the group of plants comprising *Arabidopsis*,  
3 tomato, soybean, bean, maize, wheat, and rice.

1           17. The plant cell of claim 14, said plant pathogen  
2 being *Pseudomonas syringae*.

1           18. The plant cell of claim 13, wherein said plant  
2 cell further contains an *avrRpt2* gene operably linked to  
3 regulatory sequences; and  
4           wherein said regulatory sequences comprise a  
5 promoter.

1           19. The plant cell of claim 18, wherein said  
2 promoter is a constitutive promoter.

1           20. The plant cell of claim 18, wherein said  
2 promoter is inducible by one or more external agents.

1           21. The plant cell of claim 18, wherein said  
2 promoter is cell-type specific.

1           22. A transgenic plant which contains the DNA of  
2 claim 1 integrated into the genome of said plant, wherein  
3 said DNA is expressed in said transgenic plant.

1           23. A transgenic plant which contains the DNA of  
2 claim 8 integrated into the genome of said plant, wherein  
3 said DNA is expressed in said transgenic plant.

1           24. A transgenic plant generated from the plant  
2 cell of claim 18 wherein said DNA and said avrRpt2 gene are  
3 expressed in said transgenic plant.

1           25. A seed from a transgenic plant of claim 22.

1           26. A seed from a transgenic plant of claim 23.

1           27. A seed from a transgenic plant of claim 24.

1           28. A cell from a transgenic plant of claim 22.

1           29. A cell from a transgenic plant of claim 23.

1           30. A method of providing resistance to a plant  
2 pathogen in a plant, said method comprising:

3 producing a transgenic plant cell comprising the DNA  
4 of claim 1 integrated into the genome of said transgenic  
5 plant cell and positioned for expression in said plant cell;  
6 and

7 growing a transgenic plant from said plant cell  
8 wherein said DNA is expressed in said transgenic plant.

1 31. A method of detecting a resistance gene in a  
2 plant cell, said method comprising:

3 contacting the DNA of claim 1 or a portion thereof  
4 greater than about 18 nucleic acids in length with a  
5 preparation of genomic DNA from said plant cell under  
6 hybridization conditions providing detection of DNA  
7 sequences having about 50% or greater sequence identity to  
8 the sequence of Fig.2.

1 32. A method of producing an Rps2 polypeptide  
2 comprising:

3 providing a cell transformed with DNA encoding an  
4 Rps2 polypeptide positioned for expression in said cell;  
5 culturing said transformed cell under conditions for  
6 expressing said DNA; and

7 isolating said Rps2 polypeptide.

1 33. A method of providing, in a transgenic plant,  
2 resistance to a plant pathogen, said method comprising:

3 producing a transgenic plant cell comprising the DNA  
4 of claim 8 integrated into the genome of said transgenic  
5 plant cell and positioned for expression in said plant cell;  
6 and

7 growing said transgenic plant from said plant cell  
8 wherein said DNA is expressed in said transgenic plant.

1           34. A method of providing, in a transgenic plant,  
2 resistance to a plant pathogen, said method comprising:  
3           growing said transgenic plant from the plant cell of  
4 claim 18 wherein said DNA and said *avrRpt2* gene are  
5 expressed in said transgenic plant.

1           35. A method of isolating a disease resistance gene  
2 or portion thereof in plants having sequence identity to  
3 *RPS2*, said method comprising:  
4           amplifying by PCR said disease resistance gene or  
5 portion thereof using oligonucleotide primers wherein said  
6 primers  
7           (a) are each greater than 13 nucleotides in  
8 length;  
9           (b) each have regions of complementarily to  
10 opposite DNA strands in a region of the nucleotide sequence  
11 of Fig. 2; and  
12           (c) optionally contain sequences capable of  
13 producing restriction enzyme cut sites in the amplified  
14 product; and  
15           isolating said disease resistance gene or portion  
16 thereof.

1           36. A substantially pure *Rps2* polypeptide.

1           37. The polypeptide of claim 32, comprising an  
2 amino acid sequence substantially identical to an amino acid  
3 sequence shown in Fig. 2.

1           38. A vector comprising the DNA of claim 1, said  
2 vector being capable of directing expression of the peptide  
3 encoded by said DNA in a vector-containing cell.

1                   39. A vector comprising the DNA of the *avrRpt2* gene  
2 operably linked to regulatory sequences wherein said  
3 regulatory sequences comprise a promoter.

1                   40. A vector comprising the DNA of claim 1 and the  
2 DNA of the *avrRpt2* gene operably linked to regulatory  
3 sequences wherein said regulatory sequences comprise a  
4 promoter.

1                   41. A substantially pure oligonucleotide comprising  
2 the sequence:

3                   5' GGNATGGGNNGNNTNGGNAARACNAC 3', wherein N is A, T,  
4 G, or C; and R is A or G.

1                   42. A substantially pure oligonucleotide comprising  
2 the sequence:

3                   5' NARNGGNARNCC 3', wherein N is A, T, G or C; and R  
4 is A or G.

1                   43. A substantially pure oligonucleotide comprising  
2 the sequence:

3                   5' NCGNGWNGTNAKDAWNCGNGA 3', wherein N is A, T, G or  
4 C; W is A or T; D is A, G, or T; and K is G or T.

1                   44. A substantially pure oligonucleotide comprising  
2 the sequence:

3                   5' GGWNTBGGWAARACHAC 3', wherein N is A, T, G or C;  
4 R is G or A; B is C, G, or T; H is A, C, or T; and W is A or  
5 T.

1                   45. A substantially pure oligonucleotide comprising  
2 the sequence:

3           5' TYGAYGAYRTBKR~~BRA~~ 3', wherein R is G or A; B is C,  
4   G, or T; D is A, G, or T; Y is T or C; and K is G or T.

1           46. A substantially pure oligonucleotide comprising  
2   the sequence:

3           5' TYCCAVAYRTCRTCNA 3', wherein N is A, T, G or C; R  
4   is G or A; V is G or C or A; and Y is T or C.

1           47. A substantially pure oligonucleotide comprising  
2   the sequence:

3           5' GGWYTBCCWYTBGCHYT 3', wherein B is C, G, or T; H  
4   is A, C, or T; W is A or T; and Y is T or C.

1           48. A substantially pure oligonucleotide comprising  
2   the sequence:

3           5' ARDGCVARWGGVARNCC 3', wherein N is A, T, G or C;  
4   R is G or A; W is A or T; D is A, G, or T; and V is G, C, or  
5   A.

1           49. A substantially pure oligonucleotide comprising  
2   the sequence:

3           5' ARRTTRTCRTADSWRAWYTT 3', wherein R is G or A; W  
4   is A or T; D is A, G, or T; S is G or C; and Y is C or T.

1           50. A recombinant plant gene comprising the DNA  
2   sequence:

3           5' GGNATGGGN~~GG~~NNNTNGGNAARACNAC 3', wherein N is A, T,  
4   G or C; and R is A or G.

1           51. The gene of claim 50, further comprising the  
2   sequence:

3                   5' NARNGGNARNCC 3', wherein N is A, T, G or C; and R  
4   is A or G.

1                   52. The gene of claim 51, further comprising the  
2   sequence:

3                   5' NCGNGWNGTNAKDAWNCGNGA 3', wherein N is A, T, G  
4   or C; W is A or T; D is A, G or T; and K is G or T.

1                   53. A recombinant plant gene comprising a  
2   combination of any two or more sequences of claims 50, 51,  
3   and 52.

1                   54. A substantially pure plant polypeptide  
2   comprising the amino acid sequence:

3                   Gly Xaa<sub>1</sub> Xaa<sub>2</sub> Gly Xaa<sub>3</sub> Gly Lys Thr Thr Xaa<sub>4</sub> Xaa<sub>5</sub>,  
4   wherein Xaa<sub>1</sub> is Met or Pro; Xaa<sub>2</sub> is Gly or Pro; Xaa<sub>3</sub> is Ile,  
5   Leu, or Val; Xaa<sub>4</sub> is Ile, Leu, or Thr; and Xaa<sub>5</sub> is Ala or  
6   Met.

1                   55. A substantially pure plant polypeptide  
2   comprising the amino acid sequence:

3                   Xaa<sub>1</sub> Xaa<sub>2</sub> Xaa<sub>3</sub> Leu Xaa<sub>4</sub> Xaa<sub>5</sub> Xaa<sub>6</sub> Asp Asp Xaa<sub>7</sub> Xaa<sub>8</sub>,  
4   wherein Xaa<sub>1</sub> is Phe or Lys; Xaa<sub>2</sub> is Arg or Lys; Xaa<sub>3</sub> is Ile,  
5   Val, or Phe; Xaa<sub>4</sub> is Ile, Leu, or Val; Xaa<sub>5</sub> is Ile or Leu;  
6   Xaa<sub>6</sub> is Ile or Val; Xaa<sub>7</sub> is Ile, Leu, or Val; and Xaa<sub>8</sub> is  
7   Asp or Trp.

1                   56. A substantially pure plant polypeptide  
2   comprising the amino acid sequence:

3                   Xaa<sub>1</sub> Xaa<sub>2</sub> Xaa<sub>3</sub> Xaa<sub>4</sub> Xaa<sub>5</sub> Thr Xaa<sub>6</sub> Arg,  
4   wherein Xaa<sub>1</sub> is Ser or Cys; Xaa<sub>2</sub> is Arg or Lys; Xaa<sub>3</sub> is Phe,  
5   Ile, or Val; Xaa<sub>4</sub> is Ile, or Met; Xaa<sub>5</sub> is Ile, Leu, or Phe;  
6   Xaa<sub>6</sub> is Ser, Cys, or Thr.

1           57. A substantially pure plant polypeptide  
2 comprising the amino acid sequence:

3           Gly Leu Pro Leu Xaa<sub>1</sub> Xaa<sub>2</sub> Xaa<sub>3</sub> Xaa<sub>4</sub>,  
4 wherein Xaa<sub>1</sub> is Thr, Ala, or Ser; Xaa<sub>2</sub> is Leu or Val; Xaa<sub>3</sub>  
5 is Ile, Val, or Lys; and Xaa<sub>4</sub> is Val or Thr.

1           58. A substantially pure plant polypeptide  
2 comprising the amino acid sequence:

3           Xaa<sub>1</sub> Xaa<sub>2</sub> Ser Tyr Xaa<sub>3</sub> Xaa<sub>4</sub> Leu,  
4 wherein Xaa<sub>1</sub> is Lys or Gly; Xaa<sub>2</sub> is Ile or Phe; Xaa<sub>3</sub> is Asp  
5 or Lys; and Xaa<sub>4</sub> is Ala, Gly, or Asn.

1           59. A method of isolating a disease-resistance gene  
2 or fragment thereof from a plant cell, comprising:

3           (a) providing a sample of plant cell DNA;  
4           (b) providing a pair of oligonucleotides having  
5 sequence homology to a conserved region of an RPS disease-  
6 resistance gene;

7           (c) combining said pair of oligonucleotides  
8 with said plant cell DNA sample under conditions suitable  
9 for polymerase chain reaction-mediated DNA amplification;  
10 and

11           (d) isolating said amplified disease-resistance  
12 gene or fragment thereof.

1           60. The method of claim 59, wherein said  
2 amplification is carried out using a reverse-transcription  
3 polymerase chain reaction.

1           61. The method of claim 59, wherein said reverse-  
2 transcription polymerase chain reaction is RACE.

1               62. A method of identifying a plant disease-  
2 resistance gene in a plant cell, comprising:  
3               (a) providing a preparation of plant cell DNA;  
4               (b) providing a detectably-labelled DNA sequence  
5 having homology to a conserved region of an RPS gene;  
6               (c) contacting said preparation of plant cell DNA  
7 with said detectably-labelled DNA sequence under  
8 hybridization conditions providing detection of genes having  
9 50% or greater sequence identity; and  
10              (d) identifying a disease-resistance gene by its  
11 association with said detectable label.

1               63. The method of claim 62, wherein said DNA  
2 sequence is produced according to the method of claim 59.

1               64. The method of claim 62, wherein said  
2 preparation of plant cell DNA is isolated from a plant  
3 genome.

1               65. A method of isolating a disease-resistance gene  
2 from a recombinant plant cell library, comprising:  
3               (a) providing a recombinant plant cell library;  
4               (b) contacting said recombinant plant cell library  
5 with a detectably-labelled gene fragment produced according  
6 to the method of claim 59 under hybridization conditions  
7 providing detection of genes having 50% or greater sequence  
8 identity; and  
9               (c) isolating a member of a disease-resistance gene  
10 by its association with said detectable label.

1               66. A method of isolating a disease-resistance gene  
2 from a recombinant plant cell library, comprising:

3 (a) providing a recombinant plant cell library;  
4 (b) contacting said recombinant plant cell library  
5 with a detectably-labelled oligonucleotide of any of claims  
6 41-49 under hybridization conditions providing detection of  
7 genes having 50% or greater sequence identity; and  
8 (c) isolating a disease-resistance gene by its  
9 association with said detectable label.

1               67. A recombinant plant polypeptide capable of  
2 conferring disease-resistance wherein said plant polypeptide  
3 comprises a P-loop domain or nucleotide binding site domain.

1               68. The recombinant plant polypeptide of claim 67,  
2 wherein said polypeptide further comprises a leucine-rich  
3 repeating domain.

1               69. A recombinant plant polypeptide capable of  
2 conferring disease-resistance wherein said plant polypeptide  
3 contains a leucine-rich repeating domain.

1           70. A plant disease-resistance gene isolated  
2 according to the method comprising:  
3           (a) providing a sample of plant cell DNA;  
4           (b) providing a pair of oligonucleotides having  
5 sequence homology to a conserved region of an RPS disease-  
6 resistance gene;  
7           (c) combining said pair of oligonucleotides with  
8 said plant cell DNA sample under conditions suitable for  
9 polymerase chain reaction-mediated DNA amplification; and  
10          (d) isolating said amplified disease-resistance gene  
11 or fragment thereof.

1           71. A plant disease-resistance gene isolated  
2 according to the method comprising:  
3           (a) providing a preparation of plant cell DNA;  
4           (b) providing a detectably-labelled DNA sequence  
5 having homology to a conserved region of an RPS gene;  
6           (c) contacting said preparation of plant cell DNA  
7 with said detectably-labelled DNA sequence under  
8 hybridization conditions providing detection of genes having  
9 50% or greater sequence identity; and  
10          (d) identifying a disease-resistance gene by its  
11 association with said detectable label.

1           72. A plant disease-resistance gene according to  
2 the method comprising:  
3           (a) providing a recombinant plant cell library;  
4           (b) contacting said recombinant plant cell library  
5 with a detectably-labelled gene fragment produced according  
6 to the method of claims 41-49 under hybridization conditions  
7 providing detection of genes having 50% or greater sequence  
8 identity; and  
9           (c) isolating a disease-resistance gene by its  
10 association with said detectable label.

1           73. A method of identifying a plant disease-  
2 resistance gene comprising:  
3           (a) providing a plant tissue sample;  
4           (b) introducing by biolistic transformation into  
5 said plant tissue sample a candidate plant disease-  
6 resistance gene;  
7           (c) expressing said candidate plant disease-  
8 resistance gene within said plant tissue sample; and

9 (d) determining whether said plant tissue sample  
10 exhibits a disease-resistance response, whereby a response  
11 identifies a plant disease-resistance gene.

1                   74. The method of claim 73, wherein said plant  
2 tissue sample comprises leaf, root, flower, fruit, or stem  
3 tissue.

1                   75. The method of claim 73, wherein said candidate  
2 plant disease-resistance gene is obtained from a cDNA  
3 expression library.

1                   76. The method of claim 73, wherein said disease-  
2 resistance response is the hypersensitive response.

1                   77. A plant disease-resistance gene isolated  
2 according to the method comprising:

3 (a) providing a plant tissue sample;

4 \_\_\_\_\_ (b) introducing by biolistic transformation into  
5 said plant tissue sample a candidate plant disease-  
6 resistance gene;

7 (c) expressing said candidate plant disease-  
8 resistance gene within said plant tissue sample; and

9 (d) determining whether said plant tissue sample

10 exhibits a disease-resistance response, whereby a response  
11 identifies a plant disease-resistance gene.

1                   78. A purified antibody which binds specifically to  
2 an rps family protein.

1                   79. A DNA sequence substantially identical to the  
2 DNA sequence shown in Figure 12.

1           80. A substantially pure polypeptide having a  
2 sequence substantially identical to a Prf amino acid  
3 sequence shown in Figure 5, (A or B).

---